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EVIDENCE FOR ENVIRONMENTAL FACTORS, INCLUDING PRESENCE OF HEAVY METALS, INVOLVED IN CONVERTING POTENTIAL PATHOGENS TO A VIABLE BUT NONCULTURABLE STATE IN RIVER AND ESTUARINE WATER

Jafrul A.K. Hasan
Anwarul Huq
Rita R. Colwell

UNIVERSITY OF MARYLAND
College Park, MD 20742

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Hasan, Jafrul A.K.; Huq, Anwarul; and Colwell, Rita R.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Department of Microbiology
University of Maryland
College Park, MD 20742

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13. ABSTRACT (Maximum 200 words)
A possible effect of heavy metals on the presence of Aeromonas spp. in polluted waters was observed when chemical analyses were performed on 14 water samples. Aeromonas spp. were isolated in 13 (93%) of the 14 samples where the concentration of copper and zinc was lower than 80 µg/L. No Aeromonas spp. were isolated in samples where copper and zinc concentrations were 130 µg/L. We were able to isolate Aeromonas spp. from the same site 1 week earlier when the concentration of copper and zinc was 50 µg/L and 40 µg/L, respectively. These results suggest that Aeromonas spp. may enter a viable, but nonculturable state as a response to toxic effects of copper and zinc in polluted waters.

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PREFACE

This study was supported by the National Oceanic and Atmospheric Administration (NOAA) grant NA 86 ADD.SG 066,10. This work was started in August 1989 and completed in September 1990.

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EVIDENCE FOR ENVIRONMENTAL FACTORS, INCLUDING PRESENCE OF HEAVY METALS, INVOLVED IN CONVERTING POTENTIAL PATHOGENS TO A VIABLE BUT NONCULTURABLE STATE IN RIVER AND ESTUARINE WATER

1. INTRODUCTION

Motile aeromonads are ubiquitous, waterborne microorganisms that have been implicated as the causative agents of wound infections, severe septicemia; and diarrheal diseases.[2,3] Aeromonas infections are not uncommon following direct exposure to water harboring this microorganism.[4]

Recent studies have shown the sensitivity of aeromonads to specific concentrations of copper and zinc commonly found in drinking and natural waters. The toxic effect of copper and, to a lesser extent, zinc on Escherichia coli suspended in double distilled water was reported thirty-six years ago.[5] More recently, Domek, et al.[6], described sublethal injury or loss of viability of coliform bacteria caused by copper in drinking water in concentrations as low as 25 ug/Ml. Wells[7] studied the effect of various piping materials on bacterial viability and showed that a strain of Aeromonas spp. was more sensitive to copper than other Gram-negative bacteria (e.g., E. coli, and Pseudomonas aeruginosa) by a factor of 10. Versteeg, et al.[8], recently observed a strong negative relationship between number of Aeromonas and total copper concentration. More importantly, they found that, at concentrations above 100 ug/L, virtually no aeromonads were detected in drinking water samples.

2. MATERIALS AND METHODS

2.1 Experimental Design.

In a long term study presently underway in our laboratory, water samples are being collected at sites of diving training and operations to determine microbiological hazards to professional divers exposed to polluted waters. The water samples are analyzed for a variety of physical and chemical parameters. In addition, further investigations were made to determine the effect of heavy metals, including copper, zinc, iron, manganese, molybdenum, and potassium, on the presence of Aeromonas spp. in polluted waters. A total of 14 water samples were collected from different diving sites in Maryland, Pennsylvania and Washington, D.C. between August 1989 - October 1990. The samples were analyzed for the presence of Aeromonas spp. and their correlation, if any, with heavy metals in the water. This work comprises on-going research to determine microbiological hazards associated with diving in polluted waters, as mentioned above.

2.2 Bacteriological Methods.

Conventional biochemical tests[3] were performed to identify Aeromonas spp. from polluted waters, with a slight modification of a method for isolation and identification which was developed in our laboratory.

2.3 Chemical Methods.

All of the heavy metals, including copper, zinc, iron, manganese, molybdenum, and potassium, were analyzed spectrophotometrically using a Model DR/2000 spectrophotometer, HACH Company, Loveland, CO., employing simplified test procedures, according to the manufacturer's recommendations.

3. RESULTS

Aeromonads were isolated in 13 (93%) of the 14 samples (Table 1), where concentrations of copper and zinc were lower than 80 ug/L. Aeromonas spp. were not isolated in one sample (C5) in which copper and zinc concentrations were 130 ug/L. We were able to detect Aeromonas spp. from the same site (C4) one week earlier, when the concentration of copper and zinc was 50 ug/L and 40 ug/L, respectively (Figures 1 and 2). No other relationship could be established between the presence or absence of aeromonads and other heavy metals, including iron, manganese, molybdenum, or potassium (Figures 3, 4, 5, and 6).

4. DISCUSSION

Earlier studies have shown that heavy metals and toxic chemicals may induce a viable but not culturable state in selected bacterial species.[9,10] Gram-negative bacteria, including Aeromonas spp., were shown to go undetected by conventional bacteriological procedures in laboratory-based experiment.[9] A recent study conducted in our laboratory to observe the effect of temperature on the survival of A. hydrophila in aquatic microcosms, has shown that these organisms enter into a non-culturable state within 10 days of incubation of 37^{o}C, while still viable (Hasan, Huq, and Colwell, manuscript in preparation).

Early work in our laboratory has shown that lack of culturability can not be used to assay for pathogenic microorganisms since, under adverse conditions such as low temperature or low nutrient concentrations, bacteria may cease to undergo cell division but remain viable. In the case of Helicobacter pylori, cells were able to be recovered as long as a year after going non-culturable (Shahamat, et al., submitted for publication).

The results of this study support the hypothesis that the culturability of _Aeromonas_ may be reduced by copper at elevated concentrations, such as occur in polluted waters. In addition, results obtained in this study also indicate that elevated concentrations of zinc may have a negative effect on culturability of _Aeromonas_ in polluted water. It is hypothesized that _Aeromonas_ enters a viable, but non-culturable state in response to elevated concentrations of copper and zinc in polluted water, without losing viability or infectivity. Laboratory studies are underway to determine the effect of copper and zinc on aeromonads in river water, as well as the salinity. Survival of _Aeromonas_ in the aquatic environment may not be accurately estimated by culturing. Direct microscopy, using fluorescent antibody staining, coupled with molecular genetic methods, e.g., gene probes, may be necessary for accurate determination of the public health safety of environmental waters.

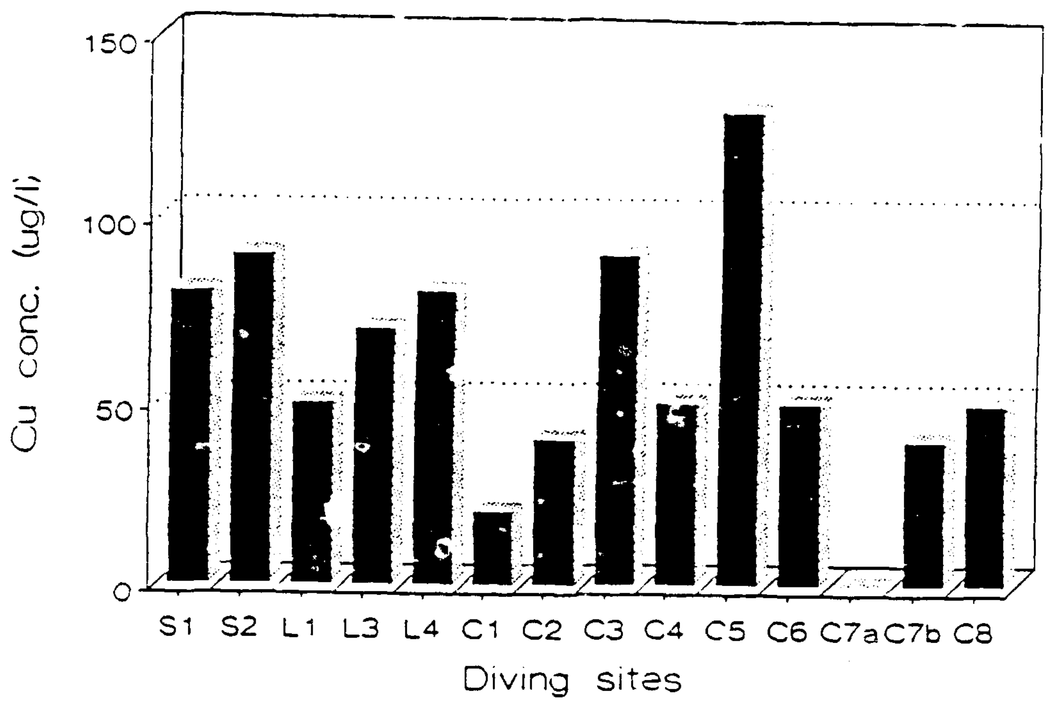

Figure 1. Concentration of copper at diving sites.

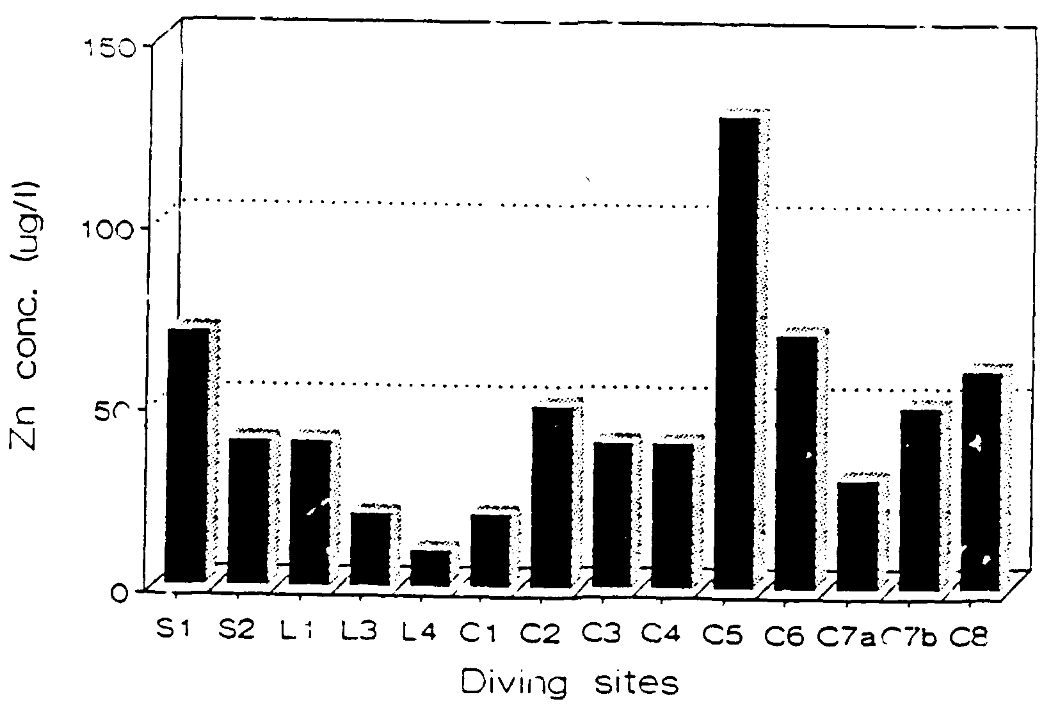

Figure 2. Concentration of zinc at diving sites.

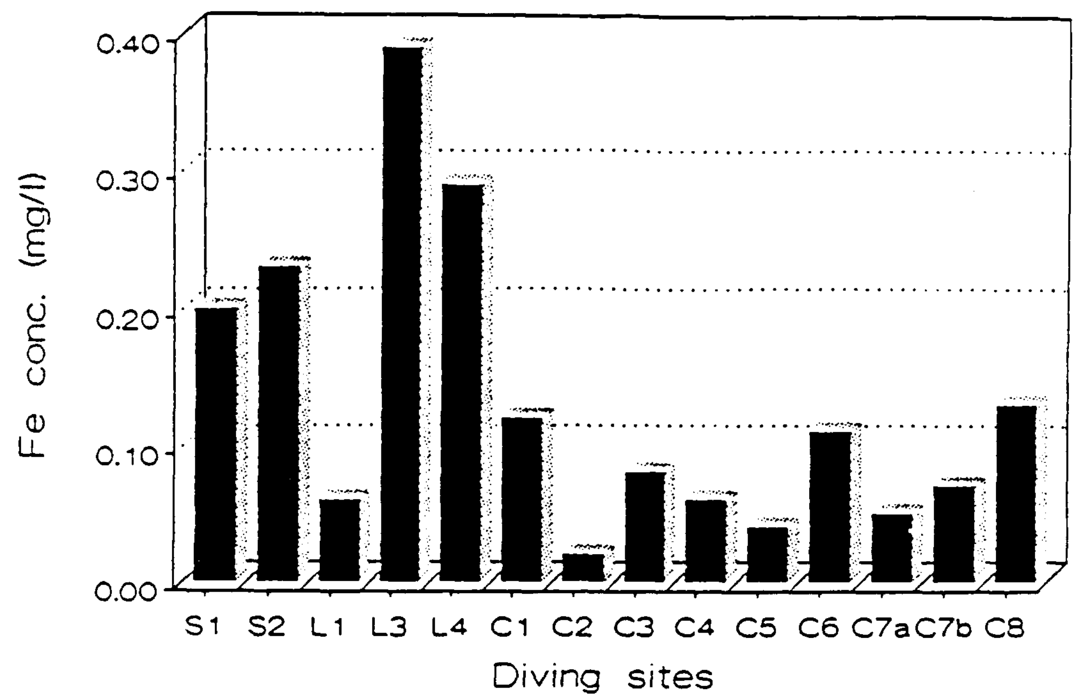

Figure 3. Concentration of iron at diving sites.

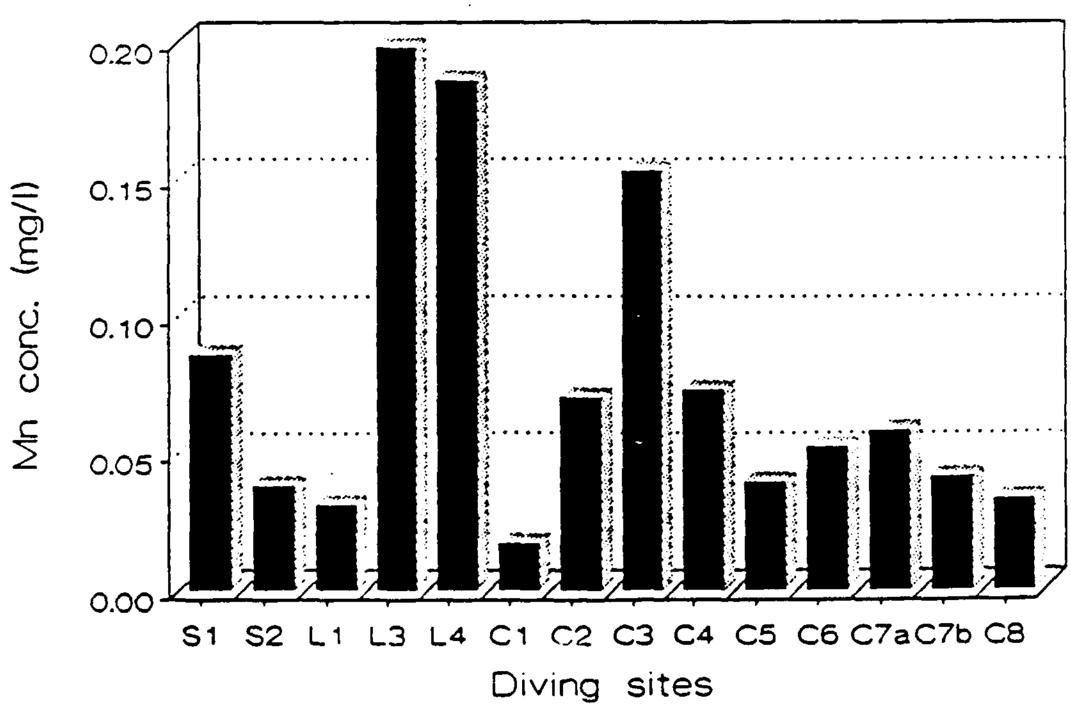

Figure 4. Concentration of manganese at diving sites.

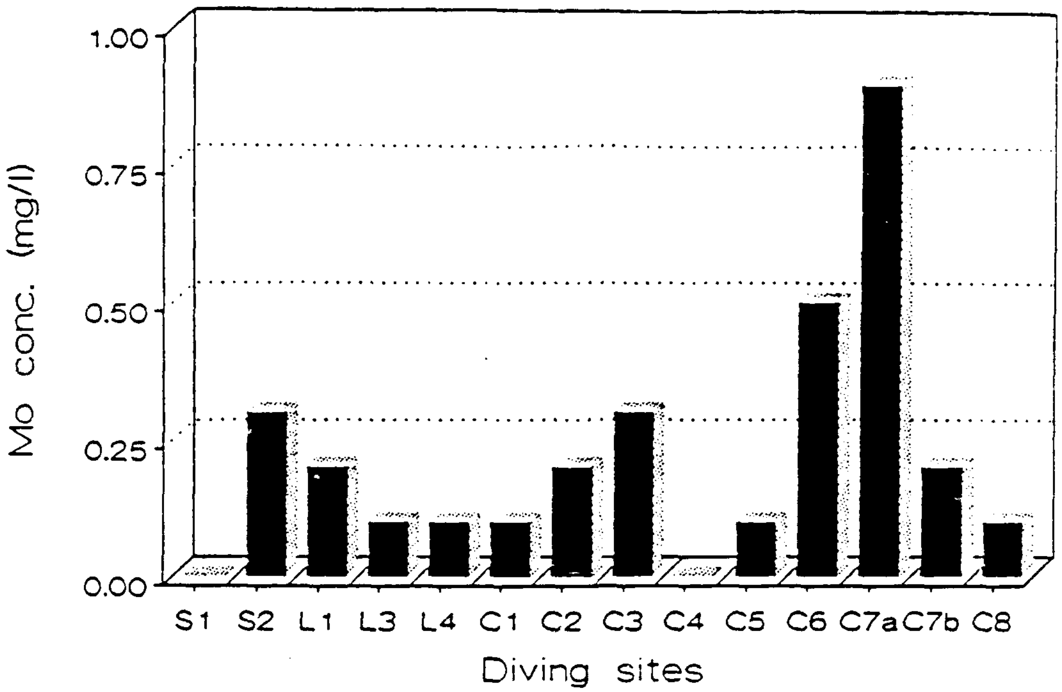

Figure 5. Concentration of molydenum at diving sites.

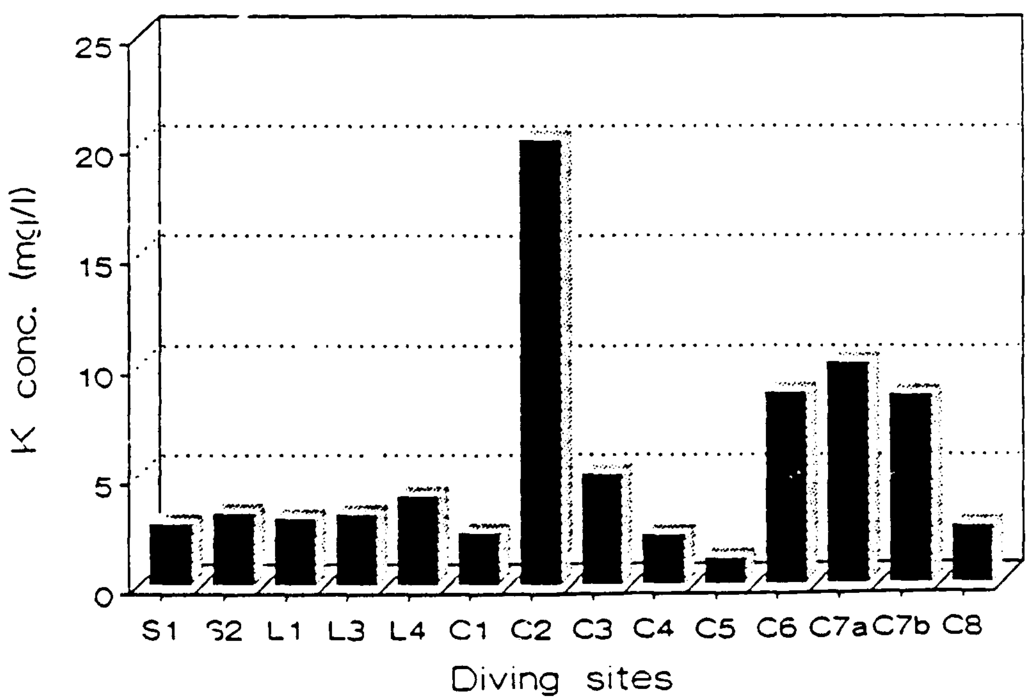

Figure 6. Concentration of potassium at diving sites.

Table. Presence of Aeromonas spp. in water samples collected from different diving sites.

Sample I.D.	Date	Site of Collection	Isolation of Aeromonas
SI	Aug 9, 1989	Anacostia River, Washington, D.C.	+
L1	Aug 16, 1989	Potomac River, Washington, D.C.	+
C1	Sep 20, 1989	Loch Raven Reservoir, Baltimore, MD	+
L3	Oct 17, 1989	4th St. Bridge, Potomac River, Washington, D.C.	+
L4	Oct 23, 1989	W.W. Bridge, Potomac River, Washington, D.C.	+
S2	Oct 25, 1989	D.C. Canal, Washington, D.C	+
C2	Nov 8, 1989	Upper Chesapeake Bay, Baltimore, MD	+
C3	Dec 6, 1989	Frog Mortor Creek, Baltimore, MD	+
C4	Apr 11, 1990	Delta, Pennsylvania	+
C5	Apr 18, 1990	Delta, Pennsylvania	-
C6	May 9, 1990	Frog Mortor Creek, Baltimore, MD	+
C7a	May 16, 1990	Seneca Creek, Baltimore, MD	+
C7b	May 16, 1990	Frog Mortor Creek, Baltimore, MD	+
C8	Sep 9, 1990	Beaver Dam Quarry, Baltimore, MD	+

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